How do bacteria interact with minerals?

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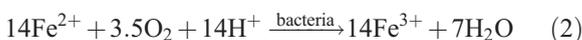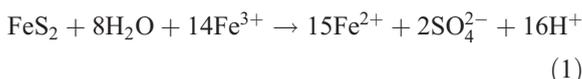
Abstract

Over the last 35 years, there has been a debate concerning the interaction of iron- and sulfur-oxidizing bacteria and sulfide minerals. Two basic positions are held. The proponents of the direct mechanism argue that the bacteria possess a specific biological mechanism to degrade the mineral and thereby gain energy directly from the sulfide mineral. On the other hand, the proponents of the indirect mechanism argue that it is the ferric ions in solution that dissolve the mineral, and the bacteria gain their energy requirements from regenerating the ferric ions. The indirect mechanism undoubtedly occurs, but proponents of the direct mechanism argue that it is not the dominant route. In other words, the debate hinges on the kinetics of each of these possible paths. The evidence presented in the published literature both for and against each of these mechanisms has been critically examined. Arguments based on stoichiometry, bacterial attachment or observations of the mineral surface cannot resolve this debate. Since the critical factor is the kinetics of each proposed path, only kinetic studies can resolve the debate. Based on the evidence of the kinetic studies that were performed, a resolution to the debate is suggested.
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Keywords: Mineral; Bacteria; Ion; Ferric ion; Mechanism; T. ferrooxidans

1. Introduction

Sulfide minerals, such as pyrite and sphalerite, are leached by ferric sulfate. This reaction produces ferrous sulfate and other dissolved products. The ferrous sulfate is re-oxidized to ferric sulfate by bacteria. These reactions can be written as:

$$FeS_2 + 8H_2O + 14Fe^{3+} \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (1)$$

$$14Fe^{2+} + 3.5O_2 + 14H^+ \xrightarrow{\text{bacteria}} 14Fe^{3+} + 7H_2O \quad (2)$$

However, in 1964 Silverman and Ehrlich (1964) proposed that this is not the only role that is played by bacteria. These researchers proposed that *Thiobacillus ferrooxidans* enhances the rate of oxidation of pyrite above that achieved by chemical reaction with ferric sulfate at the same solution conditions. This enhancement in the rate of dissolution was proposed to occur by direct microbiological action, possibly by the extracellular secretion of an enzymatic oxidant. Since the Silverman and Ehrlich proposal, different authors have made opposing claims concerning the action of *T. ferrooxidans* on pyrite. This 'direct mechanism' of bacterial dissolution of pyrite may be given by the following reaction:

$$FeS_2 + H_2O + {}^7/_2O_2 \xrightarrow{\text{bacteria}} Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (3)$$
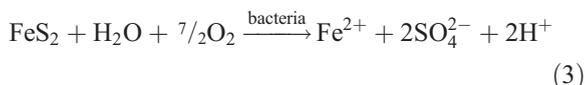
Thus, the distinguishing feature between the two proposals is the role played by ferric ions in the

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dissolution of the mineral. In the 'indirect mechanism' represented by reactions (1) and (2), the mineral is leached only by the ferric ions, whereas in the 'direct mechanism', the mineral is not leached by ferric ions, but by some biological agent. Because bacteria readily attach to surfaces, Silverman (1967) refined the classification to include three categories: the indirect mechanism, the indirect contact mechanism and the direct contact mechanism. This is illustrated in Fig. 1.

In spite of much research and debate, the questions central to the proposal of a direct mechanism remained unanswered. In this paper, the necessary and sufficient conditions are discussed in detail, and the state of the evidence for the direct mechanism is established. The results of our recent work are summarised, and a resolution of the debate is suggested.
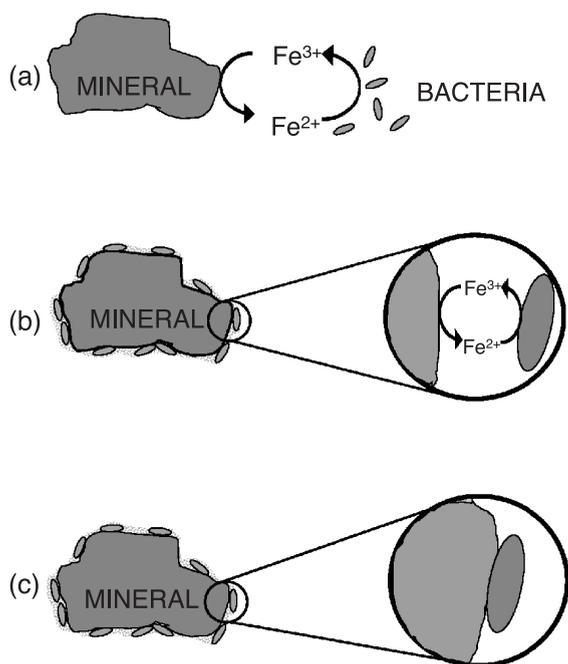

Fig. 1. The three proposed mechanisms for the action of *T. ferrooxidans* on sulfide minerals. (a) The indirect mechanism, in which bacteria oxidize ferrous ions in the bulk solution to ferric ions and ferric ions leach the mineral. (b) The indirect contact mechanism, in which attached bacteria oxidize ferrous ions to ferric ions within layer of bacteria and exopolymeric material, and the ferric ions within this layer leach the mineral. (c) The direct contact mechanism, in which the bacteria directly oxidize the mineral by biological means, without any requirement for ferric or ferrous ions.

2. Basis of the arguments for and against the direct mechanism

2.1. Necessary and sufficient conditions for the direct mechanism

It is clear from the discussion above that the sufficient condition is that the leaching of the mineral is due to a biological oxidant, not ferric ions or oxygen. The proposal that the direct mechanism exists is based on the assertion that the rate of leaching is increased by the action of the bacteria. Even though this assertion concerns the significance of the direct mechanism, rather than a requirement for its existence, it is a necessary condition for the debate to have any validity at all.

2.2. Bacterial attachment to mineral particles

The attachment of bacteria is a necessary condition for the existence of the direct contact mechanism. However, it is not a sufficient condition, because of the existence of the indirect contact mechanism, illustrated in Fig. 1b. (It may be noted that planktonic bacteria may secrete an oxidant in solution that leaches the mineral. This form of a direct mechanism has not been entertained.) Therefore, all studies and arguments that claim to have established the existence of a direct mechanism based solely on the observation of bacterial attachment to the mineral surface are rejected.

2.3. Observations of bacterial action on the surface of the mineral

A number of authors have observed that the surface of the mineral is pitted, or that there are indentations in the surface where bacteria had been attached. Hansford and Drossou (1988) observed pores in the surface of pyrite particles that were roughly the size of bacteria. However, closer examination of these pores shows that they have a hexagonal cross section, indicative of chemical etching rather than bacterial consumption of the material. Tributsch (1976) published SEM photomicrographs of the surface of PbS after bacterial leaching. These micrographs showed depressions where bacteria were attached. Tributsch concluded that the bacteria secreted a chemical carrier

that promoted the dissolution at the point of attachment. However, it was not established whether these depressions were caused by the indirect contact mechanism (Rodriguez-Leiva and Tributsch, 1988; Rojas-Chapana and Tributsch, 2001). In addition, it was not established whether the bacteria increased the rate of dissolution of the mineral.

Holmes studied the surface of single crystals of pyrite leached in the presence and absence of bacteria (Fowler et al., 2001). Observations of the surface once the bacterial biofilm had been removed indicated that that there was no difference in the patterns of leaching of the surface between the samples treated with bacteria and those treated without bacteria. In addition, Raman spectroscopy showed that there was no difference in the chemical composition of the surfaces. (Note: XPS results were found to be invalid, due to the high vacuum required by this technique, and the possible alteration of the surface by the technique. In situ studies using Raman spectroscopy are more successful.)

2.4. Identification of the biological leaching agent

Having observed the formation of depressions on the surface of sulfide minerals, Rodriguez-Leiva and Tributsch (1988) and Rojas-Chapana and Tributsch (2001) concluded that these depressions were caused by the dissolution of the mineral by a chemical carrier secreted by the bacteria. As strong supporters of the direct mechanism, they attempted to prove the existence of the direct mechanism by identifying the biological leaching agent. Rodriguez-Leiva and Tributsch (1988) argued that the leaching agent is the phospholipid found in the exopolymers, since these phospholipids acted as a surfactant for sulfur. Later, Rojas-Chapana and Tributsch (2001) identified the chemical carrier as cysteine. Although cysteine or phospholipids may be present in the exopolymers secreted onto the surface by the bacteria, these authors fail to prove a link between these chemicals and the kinetics of leaching. In particular, they would need to show that the order of reaction is affected in the same way by both the bacteria and the cysteine.

In a detailed study of the electrochemistry of the leaching of pyrite, Holmes et al. (1999) and Fowler et al. (2001) showed that for an oxidant other than ferric ions to enhance the rate of leaching, the mixed potential must increase. However, detailed measurements of the mixed potential during the leaching of pyrite show that it decreases in the presence of bacteria. This indicates that a biological oxidant does not increase the rate of the leaching of pyrite.

2.5. Arguments based on stoichiometry

An argument that has presented itself in various subtle forms is based on the stoichiometry of the growth of bacteria. For example, Konishi et al. (1994) argued that because the growth yields on various sulfide minerals was higher than the growth yield on ferrous sulfate, the direct mechanism exists and that the bacteria prefer sulfide minerals to ferrous sulfate. However, had the authors expressed their yield coefficients in units of moles rather than kilograms (since a yield coefficient is a pseudo-stoichiometric coefficient), they would have noticed that they are in proportion to the stoichiometry of the leaching reactions.

Boon (1996) measured oxygen uptake rates, and concluded that the agreement in the uptake rates for the oxidation of the mineral and of ferrous ions supports the indirect mechanism. Boon used a form of stoichiometric analysis (degree of reduction balance) to analyze the yield coefficients of bacterial growth. However, stoichiometric analysis cannot distinguish between the direct and the indirect mechanisms because all of the reactants and all of the products in these mechanisms are the same. Distinguishing between the two proposed mechanisms is not the same as choosing which is the preferred substrate for bacterial growth. The latter is a question that is amenable to stoichiometric analysis. The difficulty in this field and much of the reason for the debate is that the overall reactions are the same in both the direct and indirect mechanisms. In addition, Crundwell, (1999) showed in a response to Breed and Hansford (1999) that Boon (1996) used the same data from the measurement of the oxygen uptake rates to determine both the rate of bacterial growth and the rate of leaching. It was then argued that because the rate of leaching and the rate of bacterial oxidation of ferrous ions were the same, the direct mechanism did not exist. But they had to be the same, since they were derived from the same measurement. An independent measurement of the rate of leaching was required,

which was not provided. This illustrates the subtlety of the problem, and that many of the techniques used to study the leaching mechanism do not provide a unique answer.

2.6. Comparisons of the kinetics of leaching with and without bacteria

A necessary condition is that the action of the bacteria is to increase the rate of leaching of the mineral. This is a central question to this debate in that it concerns the significance of the debate. In spite of its importance, no detailed studies of the kinetics of leaching have been conducted, other than those by Crundwell et al. (Fowler and Crundwell, 1998, 1999; Fowler et al., 1999, 2001; Harvey and Crundwell, 1997; Holmes and Crundwell, 2000; Holmes et al., 1999).

Boon and Heijnen (1993) reviewed the kinetic data that was published in the literature for the leaching of various sulfide minerals with and without bacteria. Boon and Heijnen argued that the rate of leaching of sphalerite with bacteria is similar to that without bacteria, and that the indirect mechanism is dominant. In the case of pyrite and chalcopyrite, they argued that the direct mechanism was important, since the rate of leaching with bacteria was higher than that without bacteria. For example, they determined that the rate of oxidation of pyrite with bacteria was between 10 and 20 times the rate without bacteria. These studies were often conducted under different conditions and with different mineral samples. The origin of the mineral sample has a large effect on its rate of leaching, as Crundwell (1988) showed for sphalerite.

Boon and Heijnen (1993) argued that the minerals that dissolved more readily, such as sphalerite, were subject to the indirect mechanism, and those that dissolved more slowly, such as pyrite, were dissolved by the direct mechanism.

Other authors have attempted to study the leaching of sulfide minerals with and without bacteria in the absence of iron (Nyavor et al., 1996; Pistorio et al., 1994; Pogliani et al., 1990). However, it is known that sulfide minerals are leached by acid, albeit more slowly than by ferric ions. The product of the acid leaching is H_2S, which, once the solution is saturated, inhibits the rate of dissolution. Since the bacteria can oxidize H_2S, they remove this inhibition, and rate of dissolution in the presence of bacteria is higher than that in the absence of bacteria. Thus, this is not a useful method of determining the role of the bacteria in the leaching mechanism.

Schippers and Sand (1999) recently presented a mechanism in which they claim that the formation of various compounds of sulfur control the rate of dissolution. This work is derived from the work of Struedel (1996) on the homogeneous catalytic oxidation of H_2S. Schippers and Sand's (1999) theory does not account for the orders of the leaching reactions, and ignores the substantial body of literature that has established that the rate of leaching is determined by the electrochemical bond breaking at the surface, not by the subsequent formation of sulfur products. Since their theory does not account for the orders of reaction, they have not successfully identified the rate-determining process.

All studies that compare the rates of dissolution of a mineral in the presence and the absence of bacteria have been conducted under highly uncontrolled conditions. The leaching of the mineral by ferric ions always occurs, and the oxidation of ferrous ions by the bacteria always occurs. As a result, the trajectories of the concentrations of ferric and ferrous ions in the leaching experiment with bacteria will be different from the experiment without bacteria. If the concentrations of ferric and ferrous ions in solution are not controlled, a direct comparison of the rates of leaching with and without bacteria is of no use.

Since all of the previous studies of the rates of leaching with and without bacteria have been conducted under uncontrolled conditions, the conclusions of these studies are rejected.

3. A suggested resolution to the debate

From the survey of the previous work presented above, it is clear that prior to establishing the identity of possible biological leaching in the direct mechanism, it is critical to establish, in an unambiguous manner, whether the bacteria enhance the rate of leaching (at the same conditions in solution). No previous work has established accurate rates of leaching. Without this data, the debate has little validity.

However, this is not easy. Because the bacteria replenish the ferric ions in solution, which does not happen in the chemical leaching experiment, the concentrations of ferric and ferrous ions in the batch experiment with bacteria will be different from those in the experiment without bacteria if these concentrations are not controlled. No previous experiments had attempted to control these concentrations.

Consequently, we invented a new apparatus that controlled the conditions in solution, so that we were able to measure accurately the rates of biotic and abiotic leaching at the same solution conditions. Harvey and Crundwell (1997) presented the design of the apparatus, and Fowler (Fowler and Crundwell, 1999; Fowler et al., 1999, 2001) presented the measurements of the rate of leaching of pyrite and sphalerite.

The rate of leaching of sulfide minerals without bacteria is described by the electrochemical mechanism (Crundwell, 1988, 1999; Holmes and Crundwell, 2000; Holmes et al., 1999). Very few studies of bacterial leaching had examined the electrochemistry of the reaction, and none had used the most commonly used technique in corrosion studies: the measurement of the corrosion potential (or mixed potential). Holmes and Crundwell (2000) and Holmes et al. (1999) studied the electrochemistry of pyrite in detail. This provided new and unique data to interpret the mechanism of bacterial leaching. The results of these studies are summarised here.

3.1. Leaching of pyrite

The leaching of pyrite occurs at a higher rate in the presence of bacteria than in the absence of bacteria under all the conditions studied. Analysis of the rate of reaction as a function of concentration indicates that the order of reaction with respect to ferric ions is the same in the presence and the absence of bacteria. The reaction order is 0.5. However, the order of reaction with respect to H^+ is -0.5 in the absence of bacteria, and -0.39 in the presence of bacteria. This suggests that the presence of bacteria does not change the mechanism of leaching of pyrite, but in some manner affects the pH at the mineral surface.

It is important to appreciate the significance of the measurement of the orders of reaction, and their meaning in kinetic studies. The orders of reaction are the primary data used in establishing kinetic mechanisms in chemistry. The mechanism of bacterial leaching has always been cast in kinetic terms. No previous work in bacterial leaching has established the orders of reaction, and this is the only work that has explained these orders of reaction. It is also important not to confuse the kinetic analysis with stoichiometric analysis.

Having established that the presence of bacteria enhanced the rate of leaching but that they do not change the mechanism of leaching, Holmes et al. (1999) showed that the mixed potential decreases in the presence of bacteria, while it is constant in the absence of bacteria. A detailed mechanism of the leaching of pyrite in ferric sulfate solutions showed that both the leaching results and the mixed potential results could be explained by an increase in the pH at the mineral surface as a result of bacterial activity (Fowler et al., 1999; Holmes et al., 1999). The bacteria consume H^+ in the oxidation of ferrous ions, thus increasing the pH at the mineral surface. Since the rate of leaching of pyrite increases with increasing pH, the presence of the bacteria results in an increase in the rate of leaching. The analysis of Holmes et al. showed that this is the only explanation that accounts for the increase in the rate and the decrease in the mixed potential.

This means that the increase in the leaching rate of pyrite is a result of the indirect contact mechanism. The mechanism that explains our results is shown in Fig. 2.

3.2. Leaching of sphalerite

Fowler and Crundwell (1998, 1999) found that the rate of leaching of sphalerite with bacteria is the same as that without bacteria at low concentrations of ferrous ions. However, the rate is significantly higher than that without bacteria at high concentrations of ferrous ions. A porous layer of elemental sulfur is present on the surface of the chemically leached particles, while no sulfur is present on the surface of the bacterially leached particles. The analysis of the data using the shrinking-core model showed that the chemical leaching of sphalerite is limited by the diffusion of ferrous ions through the sulfur product layer at high concentrations of ferrous ions. The
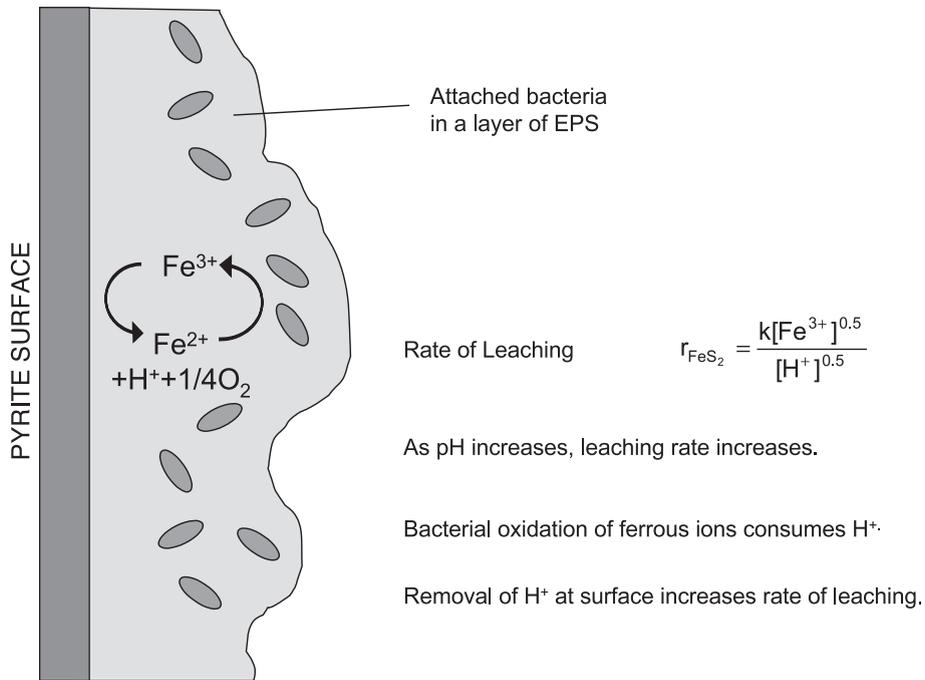
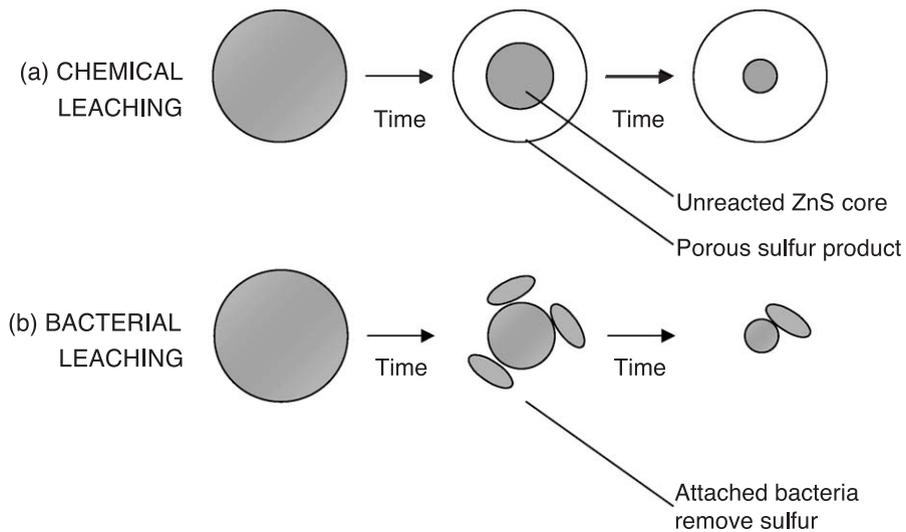Fig. 2. The mechanism of the bacterial enhancement of the rate of leaching of pyrite.

Fig. 3. A schematic diagram of a reacting sphalerite particle. (a) The chemical leaching mechanism, showing the formation of a porous layer of sulfur on the core of unreacted sphalerite. Diffusion of soluble reactant and products through the sulfur may control the rate of dissolution under some conditions. (b) The leaching mechanism in the presence of bacteria. *T. ferrooxidans* oxidizes sulfur, removing any barrier to diffusion.

analysis of the data shows that diffusion through the product layer does not limit the rate of dissolution when bacteria are present. This suggests that the action of the bacteria is to remove the sulfur formed on the particle surface, which may form a barrier to diffusion by ferrous ions. This is illustrated in Fig. 3.

4. Conclusions

Recent work performed under extremely well controlled conditions has shown that attached bacteria may increase the rate of dissolution (at the same conditions in solution) in two cases. These are: (i) if the rate of dissolution of the mineral is affected by the formation of a porous layer of sulfur by a phenomenon known as product layer diffusion, then the removal of the sulfur by the bacteria will result in an increase in the rate of leaching. (ii) If the dissolution of the mineral increases with increasing pH at the mineral surface, then the attachment of bacteria to the surface may result in an increase in the pH at the surface, which increases the rate of reaction. In both cases, detailed examination of the kinetics of leaching revealed that even though attached bacteria increased the rate of dissolution under some conditions, the mechanism of leaching was the chemical dissolution by ferric ions.

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